UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION
PREVENTION

October 28, 2014

MEMORANDUM

SUBJECT: Efficacy Review for Oxonia Active
EPA Reg. No. 1677-129
DP Barcode: 421296

FROM: Marc Rindal, Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

MR 10.28.14

THRU: Mark Perry, Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

MJP

TO: Karen Leavy, PM33
Regulatory Management Branch II
Antimicrobials Division (7510P)

APPLICANT: Ecolab, Inc.
370 Wabasha Street N
St. Paul, MN 55102

Formulations from Label

Active Ingredient(s)	% by wt.
Hydrogen Peroxide	27.5%
Peroxyacetic Acid	5.8%
Other Ingredients	66.7%
Total	100.0%

I BACKGROUND

The product, Oxonia Active (EPA Reg. No. 1677-129), a registered disinfectant (bactericide, virucide, tuberculocide), sanitizer, and deodorizer for use on hard, non-porous surfaces in institutional, industrial, commercial, and animal care environments. The label claims that the product is effective as a disinfectant in the presence of 500 ppm hard water and 5% blood serum. The applicant provided efficacy data supporting claims for effectiveness as a disinfectant against spores of *Clostridium difficile*. Studies were conducted at Ecolab, located at Ecolab Schuman Campus, 655 Lone Oak Drive, Eagan, MN 55121. The study was conducted against FF-ATH, EPA Reg. No. 1677-237. FF-ATH is 100% Oxonia Active, EPA Reg. No. 1677-129.

This data package contained a letter from the applicant to EPA (dated June 18, 2014), EPA Form 8570-4 (Confidential Statement of Formula), one study (MRID 485858-15), Statement of No Data Confidentiality Claims for the study, and the proposed label.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces such as bathroom fixtures, carts, chairs, countertops, coolers, floors, racks, refrigerators, shelves, sinks, tables, and walls. The label indicates that the product may be used on hard, non-porous surfaces including: glass, glazed porcelain, linoleum, plastic (e.g., polypropylene, polyethylene), stainless steel, tile, and vinyl. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Prepare a use solution by adding 4 ounces of the product to 8 gallons of water (i.e., a 1:256 dilution). Apply use solution with a mop, cloth, sponge, brush, scrubber, or coarse spray device, or by soaking. Allow surfaces to remain wet for 10 minutes. Remove solution and entrapped soil with a clean wet mop, cloth, or wet vacuum pickup. A pre-cleaning is required for heavily soiled surfaces.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Sporicidal Disinfectant against *Clostridium difficile*: The Agency has established interim guidance for the efficacy evaluation of antimicrobial products (e.g., dilutable products, ready-to-use products, spray products, vapor, gases, and towelettes) that are labeled for use to treat hard, non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile*. The effectiveness of such a product must be substantiated by data derived from one of the following two test methods: AOAC Method 2008.05: Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface); and ASTM E 2197: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides. Modifications to each test method will be necessary to specifically accommodate spores of *Clostridium difficile*. Because *Clostridium difficile* is an obligate anaerobe, testing should ensure adequate incubation conditions for the recovery of viable spores. Three product batches should be tested at or below the lower certified limit(s) (LCL) listed on the confidential statement of formula (CSF) of the product. The toxigenic strains, ATCC 43598, of *Clostridium difficile* must be used for testing. For towelette and spray formulations, the Agency will accept testing of the liquid

expressed directly from towelettes or collected directly from spray containers using one of the quantitative methods and conditions specified above. All products should be tested with a 3-part soil load incorporated into the test inoculum by adding 25 µl of 5% bovine serum albumin, 35 µl of 5% yeast extract and 100 µl of 0.4% mucin to 340 µl of the spore suspension. Results must show a minimum 6 log reduction of viable spores in 10 minutes or less. For towelette products, wetness determination test will be used to generate the contact time. Control carrier counts must be greater than 10^6 spores/carrier. The titer and purity of the final spore preparation must be $>10^8$ spores/mL, and >95% spores. ASTM Standard E2839 specifies procedures for achieving the 95% purity. The acid resistance of purified spores should be assessed against 2.5 M hydrochloric acid (see ASTM Standard E2839). The spores are considered acid-resistant if a log reduction of 0-2 is exhibited following 10 minutes of exposure to 2.5 M HCl.

IV SYNOPSIS OF EFFICACY STUDY

MRID 485858-15 "FF-ATH Quantitative Sporicidal Three-Step Method Against *Clostridium difficile*" for FF-ATH, by Kris Owens. Study conducted at Ecolab, Ecolab Schuman Campus. Study completion date – April 14, 2011. Study Identification Number 1100001.

This study was conducted against *Clostridium difficile* spores (ATCC 700792). Three lots (Lot Nos. J062901, J072302, and J081101) of the product, FF-ATH, were tested according to Ecolab Protocol No. 1100001 (copy provided). All three product lots were indicated to be at least 60 days old at the time of testing. Testing was conducted following Ecolab Microbiological Services SOP MS 112-01; Quantitative Sporicidal Three Step Method, created from AOAC Official Method 2008.05: Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface- Quantitative Three Step Method First Action 2008 and ASTM 2414-05 Standard Test Method for Quantitative Sporicidal Three Step Method (TSM) to Determine Sporicidal Efficacy of Liquids, Liquid Sprays & Vapors or Gases on Contaminated Carrier Surfaces to meet the efficacy data requirements based on US EPA Guidance for the Efficacy Evaluation of Products with Sporicidal Claims against *Clostridium difficile*. For each lot of product, the dilution procedure was performed using the lower limit of peroxyacetic acid to ensure that both active ingredients were at or below their lower limit. The test system used in this study was Clostridium difficile (ATCC 700792). The test system was purchased from the American Type Culture Collection (Rockville, MD) and identity was confirmed using colony morphology description and Gram Stain reaction. The test system was inoculated into four 500 mL bottles of Liver Broth and incubated anaerobically at 35±2°C for 7-10 days. After incubation, it was verified that the preparations consisted of more than 95% spores as determined by observation with a phase contrast microscope. The four bottles of culture were stirred using a sterile pipet to resuspend any spores that may have settled to the bottom. The entire 4 L were then filtered through sterile cheesecloth. Eight 50 mL centrifuge tubes were filled with 30 mL of the filtered suspension. The tubes were centrifuged at 7500xg for 20 minutes. The supernate was disposed of by pouring off and the centrifuge tubes were filled with another 30 mL of the filtered suspension. The tubes were again centrifuged at 7500 x g for 20 minutes at 20 ± 2°C. The process was repeated until the entire 4 L had been centrifuged. After the last of the suspension had been centrifuged, the eight pellets were each resuspended in 10 mL of sterile Milli-Q water and combined into two new tubes. The pellets were washed by resuspending in cold sterile

Milli-Q water and centrifuging at 7500 rpm for 20 minutes. After centrifuging, the supernate was disposed of by pouring off. The pellet was washed 4 additional times by centrifuging at 7500 x g for 20 minutes and resuspending in cold sterile Milli-Q water. The final pellets were combined and resuspended in 80 mL cold sterile Milli-Q water and stored at 2 to 8°C for less than 6 months. Prior to use in efficacy testing, the spores were enumerated by serial dilution and plating. The spore suspension may be diluted or concentrated to achieve the desired count of 10^8 to 10^9 spores/mL. Carriers used for testing were 5 x 5 x 1 mm glass carriers cut from microscope slides. Each carrier was visually screened for scratches, chips, or cracks. Any damaged or defective carriers were discarded. The carriers were rinsed once with water, three times with 95% ethyl alcohol, followed by rinsing 3 times with Milli-Q water. After the carriers were allowed to dry, they were placed in glass tubes (25 x 150 mm), 40 carriers per tube. The tubes were autoclaved at 121°C for 20 minutes on a dry cycle. Prior to testing, the necessary number of carriers were transferred to sterile plastic Petri dishes for inoculation. The carriers were inoculated by applying 10 uL of the spore suspension to the center of the carrier. The carriers were allowed to dry for a minimum of 1 hour in an open Petri dish in a biosafety cabinet followed by a minimum of 12 hours in a desiccator in a closed Petri dish at room temperature (15-30°C). To be valid, the average count should be $1.0x10^6$ - $1.0x10^7$ spores/carrier. After preparation of test substance batches, approximately 1.5 mL of each test substance batch was dispensed in microcentrifuge tubes and placed in a waterbath at 20 ± 2°C. The tubes were allowed to equilibrate for at least 10 minutes. Three carriers were tested per test substance batch. Each inoculated carrier was transferred to a microcentrifuge tube labeled Fraction A using a forceps. The Fraction A tubes were placed in a waterbath at 20±2°C. To each Fraction A tube, 400 µL test substance was added at 30 second intervals. Following the exposure period, 600 µL ice-cold Luria-Bertani (LB) broth + 0.5% sodium thiosulfate (media was kept on ice throughout testing) was added to each Fraction A tube at 30 second intervals. After agitating the tubes to thoroughly mix the components, each carrier was transferred to corresponding Fraction B tubes. Fraction B tubes were microcentrifuge tubes that contained 400 µL sterile water. Fraction A tubes were centrifuged in a microcentrifuge for 6 minutes ± 1 minute at 16,000xg. The supernate was removed and discarded without disturbing the pellet. The pellet was resuspended in 100 µL sterile water by pipetting up and down repeatedly. The entire 100 µL was plated on Brain Heart Infusion Agar with 5% Sheep's Blood (BHI Blood Agar) by spread plating. Fraction B tubes were sonicated for 5 minute ± 30 seconds using a floating microcentrifuge tube holder placed inside an ultrasonic cleaner. After sonication, 600 µL ice-cold LB broth was added to each Fraction B tube and the tubes were vortexed for approximately 1 minute. Each carrier was then transferred using flamed forceps from Fraction B tubes to corresponding microcentrifuge tubes labeled as Fraction C tubes. Fraction C tubes contained 400 µL ice-cold LB broth. After removing the carriers, the Fraction B tubes were centrifuged for 6 minutes ± 1 minute at 16,000xg. The supernate was removed and the pellet resuspended in 100 µL sterile water by pipetting up and down repeatedly. The entire 100 µL was spread plated on BHI Blood Agar. Fraction C tubes were placed on an orbital shaker at approximately 140 rpm inside an incubator for 30±2 minutes at 35±2°C. After incubation, the entire 400 µL volume of the Fraction C tubes were spread plated on BHI Blood Agar. All plates were incubated at 35 ± 2°C for 72 ± 4 hours under anaerobic conditions. The identity of a minimum of one representative colony taken from at least one plate per treatment was confirmed by Gram stain and colony morphology. The total number of spores per carrier was calculated by adding the total number of viable spores per fraction for A, B, and C. The log density was then calculated by taking the Log_{10} of the total number of spores per carrier. The log reduction (LR) of test carriers was determined

by subtracting the mean log density of test carriers from the mean log density of control carriers. The LR was calculated for each test substance batch. Controls included carrier numbers control, neutralization confirmation, HCl resistance, test system purity control and sterility control.

V RESULTS

Efficacy Results

Clostridium difficile (ATCC 700792) Endospores				
3 minute exposure period at 20°C				
Diluent 400 ppm AOAC Hard Water,				
Lot	Test Date	Survivor Log Density	Carrier Log Density	LR
J062901	02/01/11	0.00	6.37	6.37
J072302	02/01/11	0.26	6.37	6.11
J081101	02/01/11	0.00	6.37	6.37

VI CONCLUSION

1. The submitted efficacy data support the use of the product, Oxonia Active, as a Sporicide against spores of Clostridium difficile on hard nonporous surfaces with a 3 minute contract time at 20°C when prepared with 400 ppm hard water. A 6 log or greater reduction in viable spores was observed for all three product lots tested (at or below the lower certified limit for active ingredients). Control carrier counts were greater than 10^6 spores/carrier. HCl resistance, purity, and sterility controls demonstrated acceptable results.

Testing was conducted with a test method meeting Agency guidelines at that time.
Testing was conducted with a test system organism strain meeting Agency guidelines at that time.

VII RECOMMENDATIONS

1. The proposed label claims that the product, Oxonia Active, is sporicidal and kills and/or inactivates spores of *Clostridium difficile* on hard, nonporous surfaces after a 3 minute contact time with a use solution of 3 oz. per gallon of 400 ppm hard water (not tested in the presence of soil load).

Label Comments:

- Page 6: The *C. difficile* disinfection instructions must be separate from the "cleaning prior to disinfection" instructions. The *C. difficile* instructions must specify product concentration, contact time, hard water limitations and the application process. These instructions should be separate from the pre-cleaning requirements for *C. difficile*.
- Page 5: The Directions for Antimicrobial Surface Saturation should specify for "spoilage organisms."

- Page 13: Remove ...*Clostridium difficile* spore control following reference to proven "one-step" disinfectant as testing was not conducted in the presence of organic soil as indicated on page 6.